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The Effect of DDT on Learning Rate and Discrimination in Rainbow Trout, *Salmo gairdneri* (Richardson)

By

© PATRICIA G. McNICHOLL

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH

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OF MASTER OF SCIENCE

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FACULTY OF GRADUATE STUDIES AND RESEARCH

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled "The Effect of DDT on Learning Rate and Discrimination in Rainbow Trout, *Salmo gairdneri* (Richardson)" submitted by Patricia G. McNicholl in partial fulfilment of the requirements for the degree of Master of Science.

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ABSTRACT

Rainbow trout, *Salmo gairdneri*, were force fed pellets containing 0, 10 and 100% of the incipient LD50 dose of DDT with or without MS 222 anesthetization. Fish were trained to perform a simple conditioned response 48 or 72 hr after DDT administration. MS 222 did not affect learning rate, either alone or with DDT administration, at either dose. DDT at the LD50 dose significantly increased the learning rate and while the fish at 10% did not learn significantly faster than control fish, a dose response curve is indicated. The trout tested 48 or 72 hr after treatment were not significantly different, indicating the effect did not diminish over 24 hr.

Fish trained to discriminate between a bright (723 lux) and a dim (22 lux) light were tested to find the minimum difference in light intensity they could distinguish, given DDT treated food pellets at 0, 30, 50 or 80% of the LD50 dose, and retested 71 hr later. Discriminating ability decreased linearily with increasing dose, while performance and retention were unaffected. An attempt is made to explain the positive effect of DDT on learning as opposed to the negative effect on discriminating ability.

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INTRODUCTION

DDT (2,2-bis[*p*-chlorophenyl]-1,1,1-trichloroethane) is one of the best known, the most economical and the most effective of the synthetic insecticides. After its discovery as an insecticide in 1939, DDT soon became known as the "miracle insecticide" with world-wide use (O'Brien, 1967). However, even as early as 1944, its deleterious effects on fish began to be noticed (Ellis *et al.* 1944). A number of papers soon followed dealing with the acute toxicity of DDT to fish, which extend almost to the present (Ginsburg 1945; Odum and Sumerford, 1946; Washburn, 1947; Langford, 1949; Surber and Hoffman, 1949; Bry, 1952; Mayhew, 1955; Gagnon, 1958; Henderson *et al.* 1959a & b; Katz, 1961; Tarzwell, 1963; Cope 1964; Greer, 1967; Macek and McAllister, 1970; Dacre and Scott, 1971; Post and Schroeder, 1971; and many others). Paralleling these were a number of studies concerning the effects of forest spraying on fish, essentially field toxicity tests, in both Canada and the United States (Cottam and Higgins, 1946; Adams *et al.*, 1949; Cope *et al.*, 1947; Darsie, 1952; Burden, 1956; Graham and Scott, 1958; Alderdice and Worthington, 1959; Crouter and Vernon, 1959; Graham, 1960; Kerswill *et al.*, 1960; Bridges and Andrews, 1961; Cope, 1961; Warner and Fenderson, 1962; Elson, 1967; Kerswill, 1967; Kerswill and Edwards, 1967; and many others).

In recent years the trend has been towards studying sublethal and chronic effects, rather than simple lethality (e.g. King, 1962; Allison *et al.*, 1963, 1964; Burdick *et al.*, 1964; Macek 1968a & b; Buhler *et al.*, 1969, Buhler and Shanko 1970). My study deals mainly with sub-lethal effects, as these may have an important, but not

immediately apparent, effect on organisms, such as causing a change in behaviour which could, for example, make the animal more vulnerable to predation or less adept at obtaining food. The study had two main purposes. The first of these was to look at the effect of DDT on rate of learning in fish, as the literature dealing with this aspect had shown conflicting results. The second purpose was to look at the effect on discriminating ability, and because of the experimental design it was also possible to examine the effects of DDT on retention and performance of a conditioned response. None of these had been studied to any extent in fish.

The use of behavioural parameters, particularly conditioned responses, is a common means for evaluating the effects of drugs on the central nervous system, having been used in investigations of a number of pharmacological agents (e.g. Cutting *et al.*, 1959; Zakusov, 1961; McGaugh and Petrinovich, 1965), heavy metals (Weir and Hine, 1970) and pesticides (Medved *et al.*, 1964; Warner *et al.*, 1966; Anderson, 1971; Schnorr, 1972; and many others). Behavioural parameters are useful bioassay tools because behaviour is a complicated, integrated result of diverse biochemical and physiological processes, very sensitive to changes in the steady state of the organism, and easy to monitor without direct harm to the organism (Warner *et al.*, 1966).

In spite of all the work done on DDT, its mode of action is still not well known (for present theory of action, see O'Brien, 1967). According to Hayes (1959) the principal site of action of DDT in vertebrates is the central nervous system, although other areas of

the nervous system are also adversely affected. Dale *et al.*, (1963) found that the severity of DDT poisoning in rats (species not given) was directly related to its concentration in the brain indicating the importance of the brain in the toxicity of DDT. A number of changes in the electrical activity of the brain have been found with DDT administration (for review of early literature see Hayes, 1959). Cerebellar changes include increases in both the frequency and magnitude of the EEG potentials in cats (species not given) and monkeys (*Macaca mulatta*) (Crescitelli and Gilman, 1946) with some degenerative changes and cell abnormalities in the cerebellum of dogs (species not given) (Haymaker *et al.*, 1946). These and a later study by Woolley (1968) pointed to the cerebellum as the site of action of DDT. Cortical changes include increases in both frequency and amplitude of the EEG pattern, but to a lesser extent than in the cerebellum (Crescitelli and Gilman, 1946; Desi *et al.*, 1966). Changes have also been seen in the auditory and visual potentials of both the cerebellum and cerebral cortex (Wooley, 1968) and in olfactory potentials in the olfactory bulb in rats (species not given) (Wooley and Barron, 1968).

Premdas and Anderson (1963) showed that DDT quickly accumulates in the brain of fish (*Salmo salar*) exposed to DDT dissolved in their medium water (see also Holden, 1962; Grzenda *et al.*, 1970; Macek *et al.*, 1970), and central nervous system changes similar to those in mammals have been found in fish. Aubin and Johansen (1969) demonstrated an increase in the amplitude and a decrease in the frequency of the electrical activity of the brains of fish (*Carassius*

auratus) near death from DDT; however these brain activities returned to normal if the fish were returned to water which did not contain DDT.

Effects of DDT have also been found on peripheral nerves. Animals exposed to DDT are generally described as hypersensitive to stimuli (e.g. Anderson, 1971; Hatfield and Johansen, 1972); in fact, O'Brien (1967) suggests that DDT is lethal because of its effects on sensory nerves. Anderson (1968) showed that DDT increased the frequency of impulses in the lateral line of brook trout (*Salvelinus fontinalis*) but Bahr and Ball (1971) found normal lateral line discharge in rainbow trout treated with similar levels of DDT.

Because DDT has been shown to affect both the brain and peripheral nerves, it might well affect some central nervous system functions, such as learning, involving mainly the brain, and discrimination, involving both the brain and peripheral receptors.

A number of studies looking at the effect of DDT on learning in both mammals and fish have produced conflicting results. In mammals, DDT has been found either to have no effect on, or to cause delayed acquisition of a conditioned response (Khairy, 1959; Medved *et al.*, 1964; Al-Hachim and Fink, 1968; Yuhas, 1970; Sobotka 1971; Craig, 1972). The work with fish is contradictory. Anderson and Prins (1970) found that brook trout exposed to sublethal DDT doses either could not be conditioned to exhibit the propeller-tail reflex or required significantly more trials than the controls. Anderson and Peterson (1969), Jackson *et al.*, (1970) and Hatfield and Johansen (1972), all using equipment built to the same specifications

found that fish could not be conditioned if given DDT, learned as well as controls with DDT, or learned better than controls, respectively. The first purpose of the present study was to examine the effect of DDT on the rate of acquisition of a conditioned response, or learning rate, in fish.

Discrimination requires integration of central and peripheral functions in the nervous system. It is a more complex nervous process than the acquisition of a simple conditioned response and therefore might be more sensitive to sublethal levels of insecticide than a simpler response. Thompson and Lilja (1964) found a period of increased auditory acuity in rats (species not given) immediately following DDT administration, quickly followed by a decrease. Davis (1965) showed that DDT decreased the ability of bobwhite *(Colinus virginianus)* to discriminate between a red and a green light. Dieldrin, another chlorinated hydrocarbon, has been shown to decrease auditory acuity in sheep (species not given) (Elsberry, 1973). A number of other investigators have attributed their results to a possible change in discriminating ability (see Discussion). A second purpose of the present study was to look at the effects of DDT on discriminating ability, and because of the experimental design it was also possible to examine the effects of DDT on retention and performance of the conditioned response.

In all of the studies on fish cited above, DDT was admininstered in the water. However, the food chain is thought to be one of the major sources of DDT for fish in natural waters (Macek and Korn, 1970). Thus, an oral dose of DDT was given in the present

study to more closely approximate the natural situation.

MS 222 (ethyl m-aminobenzoate methanesulphonate) is widely used as a fish anesthetic (Wedemeyer, 1970). Because of its action on the central nervous system (McFarland, 1960) it may well act synergistically with DDT. It has also been shown to affect some behavioural responses (Goddard *et al.*, 1974), and central autonomic functions (Houston *et al.*, 1971) in fish. Since MS 222 was used as an anesthetic to facilitate force feeding fish throughout the study, experiments were also done to determine whether or not MS 222 had any effect on learning rate either alone or with DDT administration.

Fish were chosen as the experimental animals for a number of reasons, including most of those listed in Cutting *et al.*, (1959) as well as the relative sensitivity of fish to DDT in comparison to mammals (e.g. Ellis *et al.*, 1944); Cottam and Higgins, 1946; Adams, *et al.*, 1949). Rainbow trout (*Salmo gairdneri*) were chosen because they were readily available, are worldwide in distribution (MacCrimmon, 1971) and are intermediate in susceptibility to DDT, being more sensitive than goldfish (*Carassius auratus*) but less sensitive than some other fish such as bass (*Micropterus salmoides*) or coho salmon (*Oncorhynchus kisutch*) (Macek and McAllister, 1970). They have also been suggested (Sprague, 1970) as a standard bioassay fish, representing cool water species. Hatchery trout could have lower lethal levels than wild trout, however, as they are more susceptible to DDT than wild trout (Johnson, 1963).

MATERIALS AND METHODS

Fingerling rainbow trout were obtained from the Provincial Trout Rearing Station at Raven, Alberta, and were acclimated at 15± 1 C at a 12L-12D photoperiod with lights on at 08:00 hr for at least two weeks. The 15 C temperature was chosen because it is in their optimal temperature range for growth (Purkett, 1950) and the fish likely learn more quickly at a relatively high temperature. Also, Hester (1968) found that fish show the best discriminating ability in terms of visual contrasts at some temperature intermediate between upper and lower lethal temperatures, or near optimum. Fish were held, trained and tested at the same temperature as changing the temperature can block the conditioned response (Prosser and Farhi, 1965). All fish were held in fiberglass tanks with aerated, filtered flowing water. They were fed once daily with standard trout food (Silver Cup Trout Pellets) throughout the experiment, as DDT is more toxic to starved animals (e.g. Macek, 1968b; Wedenmeyer, 1968).

The 96 hr-LD50, which is the dose required to kill half the fish in 96 hr (as in Sprague, 1969), was determined prior to the main study. In order to determine whether delayed mortality occurred, some survivors beyond 96 hr were kept for at least a week. The DDT doses used in Parts I and II below were based on this 96 hr-LD50 dose.

The study consisted of two parts; Part I determined the effect of two levels of DDT, testing time, and MS 222 anesthetization on rate of acquisition of a conditioned response (termed rate of learning hereafter), and Part II examined the effect of three doses

of DDT on discriminating ability, retention, and performance.

Part I

The DDT levels used in this part were the 96 hr-LD50 or 1.0 toxic units (as in Sprague, 1970), 10% of the 96 hr-LD50 or 0.1 tu (toxic units), and 0 tu or control fish (hereafter referred to as controls).

Four days prior to use, fish in the weight range 15 to 40 gm were individually marked with numbered fingerling tags placed in the caudal musculature just anterior to the caudal fin. One day prior to DDT administration the fish were individually weighed (see Table 1 for group mean weights) and divided to give equal mean weights in the following groups: controls, which received solvent treated pellets; 0.1 tu group, which received a dose of 10% of the LD50 dose; and the 1.0 tu group which received the full 96 hr-LD50 dose. Each group was further subdivided into those which were anesthetized with MS 222 while being treated with DDT and those which were not. Each of these groups was again divided into three which were tested 48 or 72 hr after DDT administration. Thus a total of 12 groups of 8 fish each were tested (see Table 1).

A food pellet was prepared for each test fish so that all fish in a group received the same quantity of DDT per gram body weight. The DDT (analytical grade p,p'-DDT, 99^{+}% pure) was dissolved in petroleum ether and the appropriate volume was drawn into a 50 µl syringe. The solution was dropped slowly on to a food pellet so that as the solvent evaporated, the DDT coated and partially entered the food pellet. When fish are force-fed with such a pellet, any DDT

Table 1. Mean weights and experimental conditions for each of the 12 groups of fish in Part I. N = 8 for each group.

Group name	Mean weight (grams) ± 1 S.E.	DDT dose	Anesthetized[+]	Time of testing after treatment
C-MS-48	23.0 ± 2.5	0 tu	Yes	48 hr
C-MS-72	23.0 ± 2.9	0 tu	Yes	72 hr
C-non-48	23.0 ± 2.2	0 tu	No	48 hr
C-non-72	23.2 ± 2.8	0 tu	No	72 hr
0.1-MS-48	23.1 ± 3.1	0.1 tu	Yes	48 hr
0.1-MS-72	23.4 ± 2.9	0.1 tu	Yes	72 hr
0.1-non-48	23.1 ± 2.9	0.1 tu	No	48 hr
0.1-non-72	23.1 ± 3.2	0.1 tu	No	72 hr
1.0-MS-48	23.2 ± 3.3	1.0 tu	Yes	48 hr
1.0-MS-72	22.9 ± 2.5	1.0 tu	Yes	72 hr
1.0-non-48	23.2 ± 1.9	1.0 tu	No	48 hr
1.0-non-72	23.1 ± 2.2	1.0 tu	No	72 hr

[+]Anesthetized - Means the fish was anesthetized with MS 222 while being force fed.

which rubbed off would adhere to the fish, rather than be lost in the water where it is virtually insoluble (O'Brien, 1967). Since the volume of DDT solution was generally less than 50 µl, controls received 50 µl solvent on a food pellet. The solvent was allowed to evaporate overnight. It is unlikely there was much loss of DDT as it has an exceedingly low vapour pressure (O'Brien, 1967).

The DDT was administered by force feeding fish with the appropriate pellet. Unanesthetized fish were wrapped in a soft, wet cloth to facilitate holding. Other fish were anesthetized by placing them in a 15 C solution of 100 ppm MS 222, and leaving them until they were unable to remain upright (generally, two or three minutes) but were still respiring freely (Stage I, Houston and Woods, 1972). The pellet was placed DDT-side pointing inward into the fish's throat, and pushed with a glass rod into the stomach. The fish were then placed in fiberglass tanks with running water at 15± 1 C, all fish from a given dose in one tank. Due to a breakdown in the cold water system, and the availability of only limited cooling units, it was necessary to place all the fish in the same large tank for some of the sets. Although there may have been transfer of DDT from DDT treated fish to controls (Gakstatter and Weiss, 1967), release in fish exposed to DDT is gradual (*op cit*), and because exposure of the controls to DDT treated fish was short, in a large tank and with running water, it is unlikely that controls of these sets contained significantly more DDT than controls of other sets.

The fish were tested in a number of sets as indicated in Table 2. In each set, it was planned to include at least one fish

Table 2. Sequences of the various sets of experiments in Part I. Done Oct. '72 to Feb. '73.

Set+	9:30	10:30	11:30	12:30	13:30	14:30	15:30	16:30
1-48 hr	C MS*	C non*	0.1 MS*	0.1 non	1.0 MS	1.0 non	1.0 MS	1.0 non
2-48 hr	1.0 MS*	1.0 non	C MS	C non	0.1 MS	0.1 non	0.1 MS	0.1 non
3-48 hr	0.1 MS	0.1 non	1.0 MS	--	C MS	C non	1.0 non	--
4A-48 hr	--	--	--	--	--	--	--	1.0 MS
4B-48 hr	1.0 non	1.0 MS	0.1 non	0.1 MS	0.1 non	--	C MS	C non
5-48 hr	--	--	--	--	--	--	--	--
6/7A-48 hr	C non	0.1 MS	1.0 non	--	1.0 non	1.0 MS	C non	--
6/7B-48 hr	--	--	--	1.0 MS	--	0.1 MS	--	--
6/7C-48 hr	--	--	--	1.0 non	--	--	--	--
6/7D-48 hr	0.1 non	C MS	C non	C MS	C non	--	0.1 non	--
6/7E$_1$-48 hr	--	--	--	--	--	C MS	--	C MS
6/7E$_2$-48 hr	--	--	--	--	--	--	--	0.1 MS
1-72 hr	C MS	C non	0.1 non	0.1 MS	0.1 MS	0.1 non	1.0 MS	--
2-72 hr	--	--	C non	6 MS	0.1 non	0.1 MS	--	--
3-72 hr	0.1 non	0.1 MS	1.0 MS	--	C MS	C non	0.1 non	0.1 MS
4A-72 hr	--	--	--	--	--	--	--	1.0 non
4B-72 hr	0.1 MS	0.1 non	1.0 non	1.0 MS	1.0 MS	1.0 non	C non	C MS
5-72 hr	1.0 MS	1.0 non	--	1.0 non	--	--	1.0 non	--
6/7A-72 hr	C non	--	C MS	--	C non	1.0 MS	C MS	--
6/7B-72 hr	--	--	--	--	--	--	--	0.1 non
6/7C-72 hr	--	--	--	--	--	--	0.1 MS	C non
6/7D-72 hr	--	C MS	0.1 MS	C non	1.0 non	C MS	--	--
6/7E-72 hr	1.0 non	1.0 MS	--	0.1 non	--	--	--	1.0 MS

*Where C = a control fish, 0.1 = a fish from the 0.1 tu group and 1.0 = a fish from the 1.0 tu group, and where MS = anesthetized with MS 222 while being force fed and non = no anesthetic used.

+The first number indicates the set number, the second the number of hours after DDT administration that the fish was tested.

from each of the 12 groups (these planned sets include sets 1, 2, 3, 4B, 6/7A and 6/7D). However, due to deaths, especially in the 1.0 tu group, and the occasional inavailability of sufficient fish to complete a set, it was necessary to do some make-up sets to fill in the gaps (these include the remaining sets). A total of eight fish could be tested in a given day or 16 in the 48 and 72 hr periods of each set. The times of testing for fish from any group were staggered so that a fish from each group was tested only once in each time slot. This was done to negate the effect of time of day on conditioning (Hatfield, 1970). The times of DDT administration were also staggered to correspond to the test time 48 or 72 hr later. For each 1.0 tu test, two fish were given DDT to compensate for the expected deaths of half the fish. If both survived, one was chosen at random and tested, if one survived, it was used; and if neither survived it was necessary to repeat that dose in a later set.

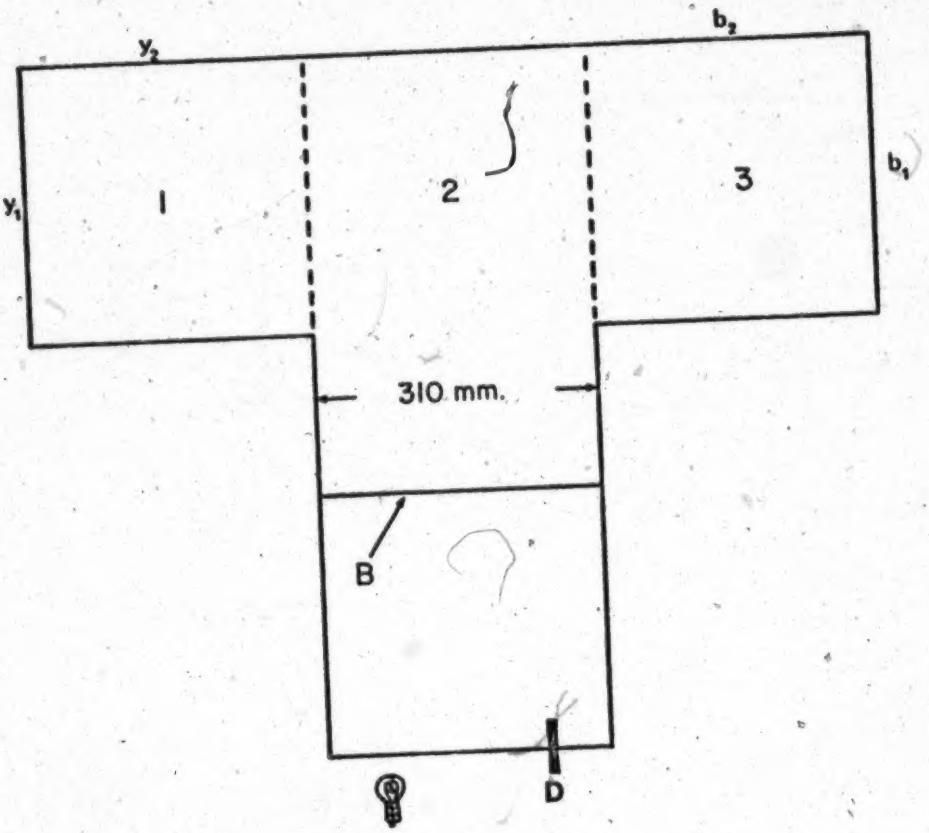
The conditioning apparatus was a T-shaped plexiglass box with dimensions as shown in Fig. 1. It was fitted with 3 stainless steel plates on the bottom, one covering each of areas 1, 2 and 3, and three identical stainless steel grids resting on plexiglass ledges, 64 mm above the plates (see Fig. 1b). The fish was placed in the water between the plates and the grids and could move freely among the three chambers. A barrier (B, Fig. 1a) made of clear plexiglass in which 5 mm diameter holes had been drilled 1 cm apart, was added to prevent the fish from seeking shelter under the rigid plexiglass tube (D, Fig 1a) which served as a drain and which was beside the light (Fig 1a). Water at 15±1C flowed into compartment 3 at the

Figure 1. The testing apparatus.

a. Top view. 1,2,3, = areas of the box, B = barrier, D = drain, ---- = arbitrary divisions between areas 1,2 and 3. The light is indicated by the symbol at the base of the T.

b. Side view of section b_1 x b_2. WL = water level, G = grid, PL = plexiglass ledge, P = plate, OT = overflow tube.

a



b

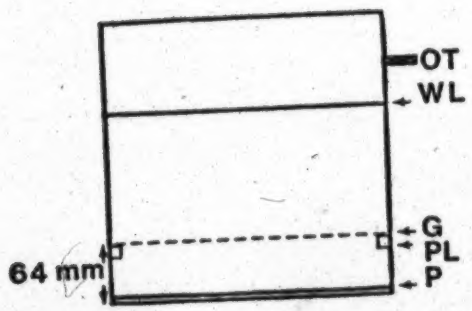


FIGURE 1.

top and flowed out the drain with additional overflow tubes provided at a depth of 228 mm on y_2, b_2 and halfway between. Two sides of compartment 1 (y_1 and y_2) were covered with yellow plexiglass sheeting, and those of compartment 3 (b_1 and b_2) with blue sheeting.

The plates and grids of each of the three compartments were connected to a switch box, which in turn was connected to an alternator, which changed the current of the DC battery which served as a power source from DC to AC. Closing one of the switches on the switch box caused a 60 cycles/sec square wave pulse of approximately 2.1 mamps/cm^2 to flow between the plate and the grid in any of the three compartments. A light connected to a second 12V battery was set to deliver 723 lux of light to the fish. The entire apparatus was in a nearly lightproof room with a 7.5 watt red light as the only illumination. The observer sat beside compartment 3.

One fish was placed in compartment 3 of the apparatus 48 or 72 hr after administration of the DDT or solvent treated food pellet and allowed to become accustomed to the apparatus for at least 10 min. It was then gently pushed with a metal rod or plastic ruler into compartment 2, and the light turned on for 10 sec. If the fish went to compartment 3, the light was turned off. If it went to 1 or stayed in 2, it was shocked by a 0.2 sec (approximately) shock and the light turned off. It was then gently pushed back into compartment 3, given a 30 sec rest, and the sequence repeated. Any fish which did not make the correct response of moving into compartment 3 in four to six of the first ten trials was discarded to eliminate fish with a strong left or right preference. Each trial

was recorded as either an error (a shock) or correct. Once a fish made ten consecutive correct responses it was considered to have reached criterion. It was then given ten more trials in which it had to make at least eight correct responses. If a fish fulfilled these two requirements it was considered to have learned the task. Total testing time was 30 to 45 min per fish.

Fish which were disorientated or lost equilibrium prior to or during the test were discarded. This occurred only in the 1.0 tu group and was taken as an indication that the fish was near death. Fish at the highest dose level appeared more sensitive to shock than fish at lower doses of DDT (as seen by Saunders, 1969), and were sometimes killed by the same shock which just caused a response in fish at other doses.

Part II

To eliminate the problem of fish dying from DDT as occurred in Part I, it was decided to use a slightly lower DDT dose as the highest dose in Part II. Thus DDT doses of 0, 0.3, 0.5 and 0.8 tu were used in Part II.

The main purpose of Part II was to look at the effect of sub-lethal doses of DDT on discrimination. The basic procedure was to train fish to discriminate between two light intensities, find the minimum difference in light intensity they could distinguish, give a DDT or solvent treated food pellet at one of the above doses, and retest. Scores on the retest also gave an indication of changes in retention and performance. According to Blackwell (vide Hester, 1968) data from forced-choice experiments are generally superior to data

from a yes-no response. Therefore the fish were trained to make one of two responses when the light was turned on, going one way in the T maze for a dim light, the other for a bright light. This type of data would be superior to that gained by having the fish move to one side when the light was bright but not move when it was dim, for example.

The apparatus was the same as that used in Part I with a few changes. A regulated power source was substituted for the batteries to give a more constant power supply so that light intensities at a given setting would not vary. Three timers were connected to the three grids so that a shock of constant duration (0.2 sec) was delivered each time. In Part I the shock had been given by a rapid flick of the switch. Extra barriers were used during discrimination training (to be explained more fully later). A large rock was placed on the grid above the barrier B (Fig. 1a) during discrimination training. The fish tended to seek cover under this rock and were thus enticed to move back into compartment 3 after each light presentation.

The light bulb was the same as that used in Part I, a 12V automobile light. It was connected to either of two variable resistors, and could be changed from one to the other by a two-way switch. Each variable resistor had a dial with a scale that could give light intensities in arbitrary units from 0 to 100 with 0 being dim (<u>not</u> off) and 100 being the brightest. The light intensity on the dim dial was varied between 0 and 50, and on the bright dial between 50 and 100. Thus by setting the two dials at the required

intensities a dim or bright light could be presented by flipping the switch, so it was not necessary to change the dial settings each time. The light energy of various wavelengths was measured in the water where the fish normally positioned themselves prior to light presentation (behind barrier B) at two light intensities, 100 on the dial (723 lux) and 50 on the dial (139 lux) and found to give an emission spectrum with greatest energy at 700 nm (see Fig. 2). The measurements were done with a glass probe attached to an ISCO Model SR Spectroradiometer. The light intensities at each setting of the variable resistor dials were found by means of a Lambda L1-185 Quantum/Radiometer/Photometer with a visual spectrum probe, the probe being placed under water in the position from which the fish usually viewed the light (behind barrier B). Dial settings 100 to 50 on the high intensity dial were measured and 50 to 0 on the low intensity dial (Fig. 3) as these were the settings actually used in discrimination testing. Room illumination was not determined; because it was a dim (7.5 W) red light (which would not tend to be filtered out by the water) located approximately 3 m from the fish it is likely the light intensity reaching the fish would be negligible.

In order to conserve time, the fish were trained to discriminate in groups of six. Preliminary tests showed that this was the largest workable group; more than that proved too difficult to count at a glance and also increased the time between light presentations. Preliminary experiments also showed that addition of one trained fish to a group of naive fish did not enhance the

Figure 2. The emission spectrum of the light used for discrimination testing.

⊙——⊙——⊙ = curve for light at 723 lux.

⊙- -⊙- -⊙ = curve for light at 139 lux.

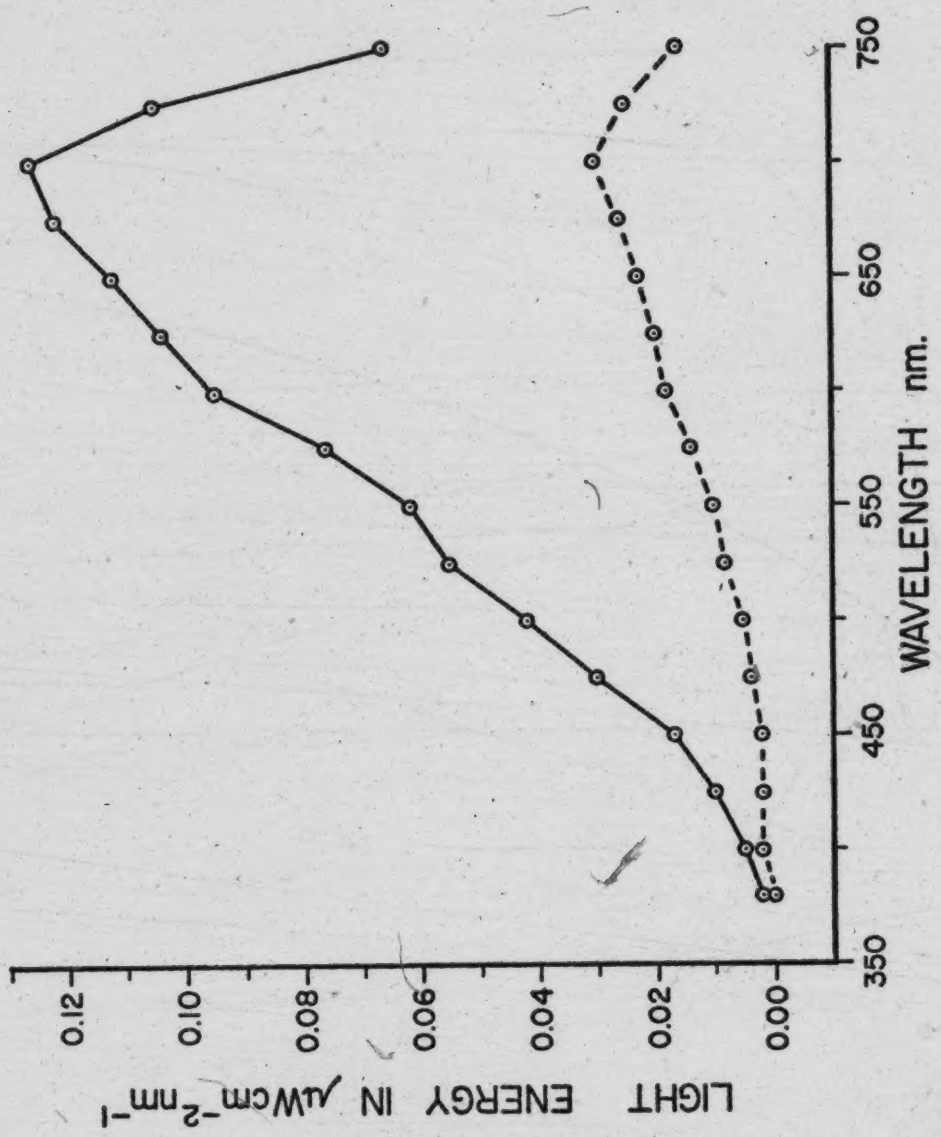

LIGHT ENERGY IN $\mu W cm^{-2} nm^{-1}$
0.00
0.02
0.04
0.06
0.08
0.10
0.12
350
450
550
650
750
WAVELENGTH nm.

FIGURE 2.

Figure 3. Light intensities at each of the settings on the variable resistor dials. Each arbitrary light intensity reading corresponds to a number on the dial.

⊖——⊖——⊖ = readings for the low light intensity dial.

⊖- -⊖- -⊖ = readings for the high light intensity dial.

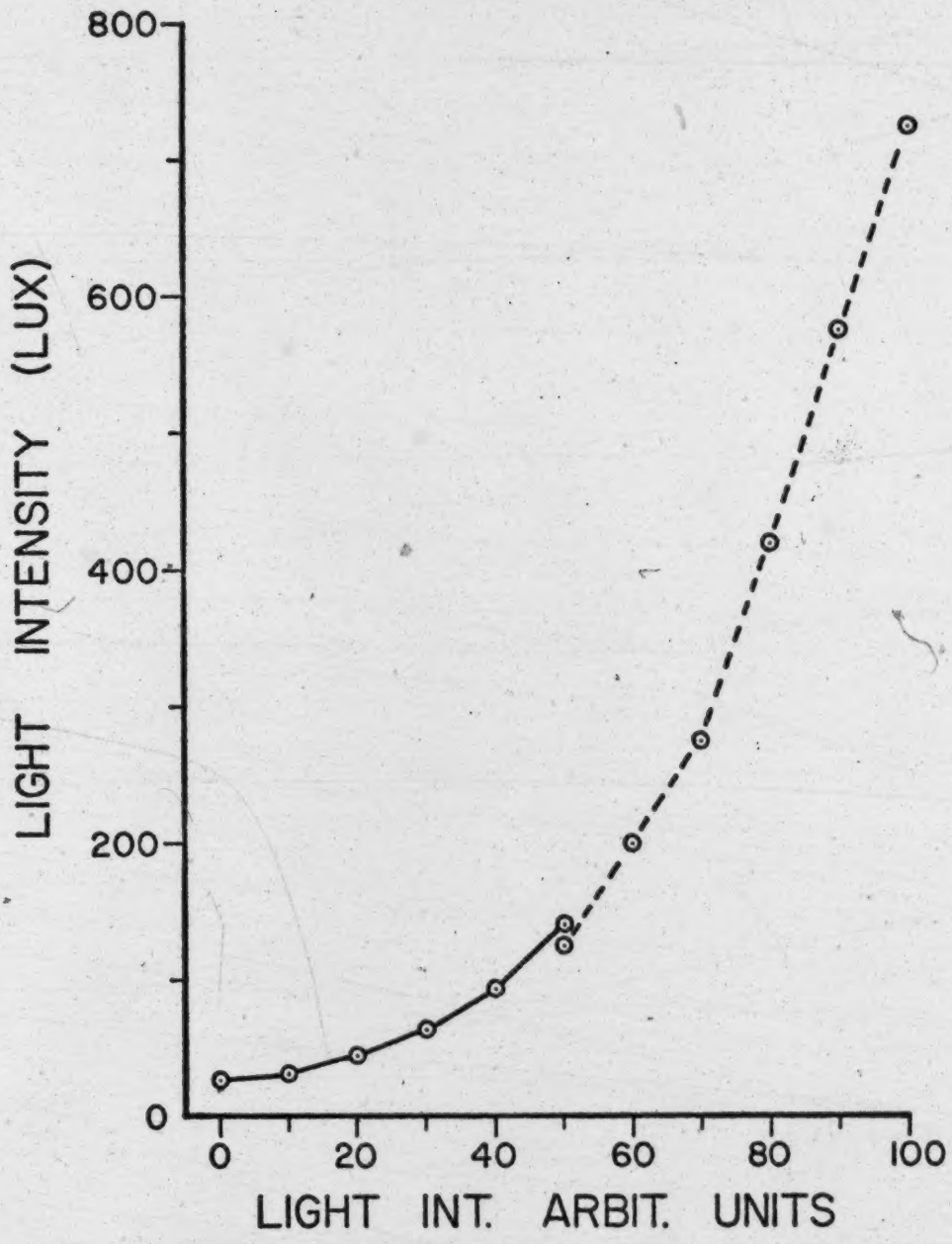

800
600
400
200
0
LIGHT INTENSITY (LUX)
0
20
40
60
80
100
LIGHT INT. ARBIT. UNITS

FIGURE 3.

group's speed of learning, while addition of one naive fish to a group of trained fish generally increased the learning rate of the one naive fish, if the fish was added early in the training sequence. Therefore, generally groups of naive fish were used, but if one fish died early in the training sessions a naive fish was added to replace it. The use of extra barriers during training also increased the learning rate. If the wrong arm of the T maze were blocked so that on presentation of the light the fish could only enter one of compartments 1 or 3, then most fish learned more rapidly which side to enter. At first they relied on the extra barriers to "show" them which way to go, but later on during training the extra barriers were not necessary and were removed. Fish were trained daily for 45 min except where otherwise noted below. Four groups were trained per day (a maximum of 24 fish) in the time interval 09:30 to 15:15, because the time period around noon is the optimal time for learning, at least in *Salmo salar* (Hatfield, 1970). The fish were trained in the following sequence (each stage represents one day of training).

Stage 1. Group Bright. The fish were trained to go to compartment 3 (Fig 1a) when the brightest light (723 lux) was presented. This training was exactly the same as in Part I except for the extra barrier barring entry to compartment 1. If all fish in the group made 20 correct responses before the 45 min training session was finished, training was stopped for that day. Due to the presence of the rock over barrier B, the fish tended to move out of compartment 3 when the light was turned off. Those which

did not move out were pushed out with a rod or startled out with a tap on the side of compartment 3. Most fish rapidly learned to move into compartment 3 when the light was turned on, and back into compartment 2 when it was turned off. The light was on for 10 sec with a 20 sec rest between light presentations.

Stage 2. A one day rest was always given between Group Bright and Group Dim. This appeared to make learning in Group Dim more rapid.

Stage 3. Group Dim. Each group was trained to go to compartment 1 when a dim (22 lux) light was presented. The extra barrier was placed in front of compartment 3. Seldom did any group respond correctly by the end of this training session. Most fish spent the greater part of the session trying to get into compartment 3. Fish also occasionally died during this training session; after a series of attempts to get into compartment 3 some fish merely stayed in compartment 2 and were shocked during every light presentation; they eventually began to fall over with each shock and were removed.

Stage 4. Group Both 1. In this session 20 bright (723 lux) light presentations were alternated with 20 dim (22 lux) presentations, with extra barriers in place where appropriate.

Stage 5. Group Both 2. Ten bright light presentations were alternated with ten dim presentations, with extra barriers in place.

Stage 6. Group Both 3. Ten presentations of each light intensity were given. If the fish were responding well, the extra barriers were then removed. Ten trials each of bright and dim light intensities were

then presented, then five of each, then random presentation of the two light intensities. Random presentation was arranged such that from one to four presentations of a given light intensity were presented in succession with the stipulation that of each ten trials, five would be dim and five bright.

Stage 7. Group Both 4. The fish were given ten presentations of the bright light, then ten of the dim light, and the extra barriers removed if they had not been removed in Group Both 3. They were then given five presentations of each light intensity, then random presentation. Near the end of this session, fish which consistently made mistakes were removed from each group until the 12 best of the 24 trained fish were left. The fish which were not used immediately were placed in a holding tank until later use. The 12 best fish were weighed, marked with fingerling tags as in Part I, and divided into groups so that three fish were used at each dose level.

Stage 8. Individual Test 1. On this day the first four fish, one from each dose, were tested individually in the apparatus at the highest and lowest light intensities (723 and 22 lux) with random light presentation and no extra barrier. They were tested at approximately the same time of day at which they would later be tested in the discrimination test. If a fish did not perform well enough here (i.e. did not get at least 80% correct in the last 20 to 30 trials) it was removed, placed in the holding tank with the others, and another fish substituted for it.

Stage 9. Individual Test 2. The second set of four fish, one from each dose level, was tested as in Individual Test 1.

Stage 10. Individual Test 3. The third set of fish was tested as in Individual Test 1.

One day prior to Pretest 1, the fish from the first set were reweighed and DDT treated pellets made up for each fish as in Part I. Fish from the second set were reweighed on Pretest 1 day and from the third set on Pretest 2 day, and their pellets made up on those days.

Stage 11. Pretest 1. The rock was removed for this and all subsequent days as it may have obstructed the fish's vision. The four fish from the first set were now tested for discriminating ability at their appropriate times. They were given five presentations of the light at 723 lux, then five at 22 lux, then three at 572 lux, three at 28 lux, two at 572 lux, two at 28 lux, then random presentation of the light intensities. The light intensities were brought gradually closer together in a series of regular steps on the variable resistor dials, giving paired light intensities of 723-22 lux, then 572-28 lux, then 419-41 lux, 273-64 lux and 200-96 lux (where 723-22 lux means the bright light was at 723 lux, the dim at 22 lux, etc.). If a fish made any mistakes during this stepwise sequence of presentation, that pair of light intensities was repeated until the fish made no mistakes at that level, then the light intensities were shifted closer together. Preliminary testing showed that most fish ceased to be able to discriminate between the two light intensities at the 200-96 lux level (a difference of 104 lux). Thus fish were expected to be able to discriminate at the 273-64 lux level and so were shocked for mistakes there but were not shocked at the 200-96

lux level, the mistakes merely being noted. If the fish made approximately half the responses wrong, this was considered to be its level of discriminating ability (as in Hester 1968). If it responded correctly or nearly so to the 200-96 lux level, the light intensities were shifted closer together to 160-118 lux. No fish could discriminate between lights at these two intensities. Once the level of discriminating ability was found the first time, the light intensities were shifted back to 572-28 lux and the sequence repeated. This time, however, as the light intensities neared the level of discriminating ability found in the first run, finer gradations of light intensity were used. For example, a fish which had failed at 200-96 lux would be given 572-28 lux, 419-41 lux, 273-64 lux, then 238-80 lux (at which he was not shocked for mistakes), then 200-96 lux. Ideally the fish would discriminate perfectly at 238-80 lux, then get half wrong at 200-96 lux. This would be an obvious score of 200-96 lux. Of course many fish did not give such sharply defined limits as this, so it was necessary to establish some arbitrary criteria. If a fish made less than 1/4 incorrect responses at a given level, it was considered that the fish could still discriminate at that level (for example, one wrong out of ten at the 238-80 lux level was fairly common and was considered to indicate discrimination). If a fish got 1/4 to 1/3 wrong inclusive, it was given a score such as 238-80 lux to 200-96 lux, if it got for example three wrong out of ten at 238-80 lux. The level of discriminating ability was taken as the difference between the two light intensities at which the fish makes half the responses wrong. If its score was 238-80 lux, this equals

a difference of 158 lux. The score on a 238-80 to 200-96 lux fish would then be the average of the two. This number - 104 lux for the 200-96 lux level, for example - essentially means that the fish has just ceased to discriminate when two lights differ by that many lux.

This testing period was run for as long as necessary to find the level of discriminating ability, up to one hour. At the end of the hour the fish was given its DDT or solvent treated food pellet as in Part I (except that all fish were anesthetized) and returned to a holding tank with the other fish of the same dose level.

Stage 12. Pretest 2. The second set of fish was tested for discriminating ability as in Pretest 1.

Stage 13. Pretest 3. The third set of fish was tested as in Pretest 1.

Stage 14. Posttest 1. Fish from the first set were retested at exactly the same time as their testing had begun in Pretest 1. Thus they were exposed to DDT for 71 hr when retesting was started. A score in lux was also found in this test, as in Pretest 1. The "change in discriminating ability" was found by subtracting the difference between the light intensities (in lux) after DDT was given from the difference in lux before DDT, for each fish. Thus if a fish scored 200-96 lux before DDT (104 lux difference) and 238-80 lux after DDT (158 lux difference) its change in discriminating ability would be 104-158 = -54 lux. Alternately if a fish went from 238-80 lux before DDT to 200-96 lux after DDT administration

it improved and its score (158-104 = 54) would show a positive sign to indicate this improvement. A score of 0 means no change; thus a fish with 200-96 lux before and after still gets the same score as a fish with 238-80 lux before and after.

Stage 15. Posttest 2. This was done as in Posttest 1, with the second set of fish.

Stage 16. Posttest 3. The third set of fish was retested as in Posttest 1.

This series was carried out with zero or one days between training sessions until Individual Test 1 was reached. Then all individual tests were done on three successive days, followed by zero or one day off, then Pretest 1 to Posttest 3 were done on six consecutive days. This was done to keep a constant time between DDT administration and retesting and to make maximal use of the trained fish.

Once this series was completed, the other trained fish which had been held for 9-10 days since the beginning of Individual Test 1 were retrained. This generally took four retraining sessions, done twice a day for two days. These fish were then individually tested, pretested, given DDT or solvent treated pellets, and posttested as in sets 1 to 3. Normally only two sets could be completed with this second series as some fish could not be conditioned and others died or became insensitive to the shock. The whole series was repeated with naive fish until completion of the experiment.

The training and testing of these fish was staggered in all doses so as to compensate for any effect of time on learning ability

(Hatfield 1970) or discriminating ability. It had originally been planned to test three fish from each dose at each of four times in the day, 09:30 to 10:30, 10:45 to 11:45, 13:00 to 14:00 and 14:15 to 15:15 (the 15 min break between is to allow the new fish to become accustomed to the apparatus. The 09:30 fish was actually put in at 09:15 to 09:20 and so on), staggering the testing such that fish of a given dose were tested at a given time every fourth set. However, due to the occasional death and fairly frequent refusal of the fish to perform during either the pretest or posttest, not all sets were completed each time (see Table 3 for actual series of sets) and the missing fish were replaced in the make-up sets M1 to M6 (I^2 was an extra set done by mistake). Some fish performed well in the individual test but would not perform in the pretest. Why they did this I do not know. It is not uncommon, however, in fish training; Hester (1968) mentions the same problem. Most of the blank spaces in the planned sets in Table 3 represent this failure to perform on the pretest. There were some fish that performed well on the pretest but failed to perform in the posttest, as well. This did not appear to be dose dependent, occurring equally in all doses including controls. It is apparent from Table 3 that many of these failures occurred in the 14:15 to 15:15 time period. It was observed in the training sessions that during this same time period the fish did not learn as well as earlier in the day. Perhaps this is reflected in their performance once they have learned the task. The 14:15 to 15:15 time is outside the optimum time period for learning of 11:00 to 14:00 given by Hatfield (1970) but so is the 09:30 to 10:30 period which shows no

Table 3. The sets done for Part II. One fish from each dose tested where indicated. Done Nov. '73 to Apr. '74.

Set	Time of testing 9:30-10:30	10:45-11:45	13:00-14:00	14:15-15:15
A	control	0.3 tu	0.5 tu	0.8 tu
B	0.3 tu	0.5 tu	0.8 tu	control
C	0.5 tu	--	--	0.3 tu
D	--	control	0.3 tu	0.5 tu
E	control	0.3 tu	0.5 tu	--
F	0.3 tu	0.5 tu	0.8 tu	--
G	0.5 tu	--	control	--
H	--	control	0.3 tu	--
I[1]	control	0.3 tu	0.5 tu	0.8 tu
M1	--	--	--	control
I[2]	control	0.3 tu	--	0.8 tu
J	0.3 tu	--	0.8 tu	--
K	0.5 tu	0.8 tu	control	0.3 tu
L	0.8 tu	control	0.3 tu	0.5 tu
M2	0.8 tu	--	control	0.3 tu
M3	0.8 tu	0.8 tu	0.5 tu	--
M4	--	0.5 tu	--	0.8 tu
M5	--	0.8 tu	--	control
M6	--	--	--	0.5 tu

greater number of failures than the other two periods. Hatfield (*op cit*) does not give the times his lights came off and on, but it is likely 12:00 hr was his midday as he did experiments at 09:00 and 15:00 and was on an 8 hr L-12 hr D photoperiod. Midday in the present experiment would be at 14:00 hr, near the time when the fish did not perform as well.

Disease, particularly fin rot, presented a fairly serious problem throughout most of Part II. Fish in sets A to E were generally healthy, and received no treatment. In sets F to M1, the fish showed signs of fin rot and so were given one treatment with 8 mg/gal $KMnO_4$ for half an hour prior to the first pretest. Two fish died from fin rot in this series. A series between F to M1 and I_2 to K nearly all died from fin rot so the survivors were not tested. New fish were obtained from the Trout Rearing Station for the remaining sets; all showed early signs of fin rot when brought in from the station. In sets I_2 to K, the fish were given a series of treatments over a three day period prior to training, including a 20 sec dip (excluding gills and head) in 1:1000 aqueous merthiolate, two treatments of 1.5% salt for 1 1/2 hr, and 10 min in 16 mg/gal $KMnO_4$. Tanks were also disinfected. The fish in this series still developed some fin rot so it was necessary to continue the merthiolate dip for the last week and clip the diseased tissue from the tail and other fins. The level of discriminating ability was not as good in these fish as in other sets (but because each fish was compared to its own score in the retest, the relationship did not change). It was decided to dispense with the $KMnO_4$ treatment in the remaining

series as it may have been discoloring the cornea and thus interfering with vision. So for fish in sets L to the end, the fish were fin clipped prior to training to remove any diseased tissue and each fish was dipped daily in merthiolate. This appeared to cure the disease as new, undiseased tissue was growing back on all fins by the end of the series. The disadvantage of the latter treatment was that it involved daily anesthetization to facilitate handling. It was considered better to anesthetize and dip the fish from the gills back than to immerse the whole fish and risk gill or eye damage from the solution. The problem with disease is not unexpected; the fish were daily exposed to a number of stresses during training, particularly that of handling, and the added stress of DDT which can make fish more susceptible to other diseases (Cope, in Chichester 1965; Macek 1968b; Sprague 1971). Treating different sets of fish differently could not be avoided as some method had to be found to keep the fish disease-free. The treatment for fin rot throughout does add an extra variable but without it the fish would not have survived. Because one fish from each dose was tested in each set (or planned) any effects of treatment would be on all fish at all doses, which should minimize any effect of treatment.

RESULTS

The 96 hr-LD50 for a single oral dose of DDT was found from the lethality tests to lie between 0.03 and 0.04 mg DDT/gm body weight (Appendix 2). Fish generally died 24 to 48 hr after DDT administration. No delayed mortality occurred, thus the 96 hr-LD50 is likely the incipient LD50 (as in Sprague 1970). It was decided to use 0.03 mg DDT/gm body weight as the LD50 dose in Part I and adjust the toxic units later if necessary. Use of this dose in the 1.0 tu group in Part I resulted in an overall death rate of 44.94% (40 of 89 fish) which is not significantly different from 50% ($Z = 0.79$). Therefore 0.03 mg DDT/gm body weight is the LD50 dose. The doses in Part II were also calculated on the basis of this figure.

Part I

The number of trials and the number of errors (shocks) to criterion for each fish were recorded and group means were determined (see Table 4). An analysis of variance (ANOVA) done on the 12 groups showed no significant difference ($p > 0.01$) between MS 222 treated and non MS 222 treated fish, nor between fish tested 48 or 72 hr after DDT administration, for either of the measures of learning rate, trials or errors to criterion. The only significant difference found for both these measures was in the effect of dose ($F = 11.2$ at 2, 77df, $p < 0.001$ for trials to criterion, $F = 14.3$ at 2, 77df, $p < 0.001$ for errors to criterion). Because there were no significant differences within the doses in terms of anesthetic or time of testing, all data from a given dose level were pooled (Appendix 3). A Duncan's Multiple Range Test showed that in terms of both trials and errors to criterion, controls were not significantly different from the 0.1 tu

Table 4. Mean number of trials and errors to criterion in each of the 12 groups.

Group	N[1]	$\bar{x}$ trials ± 1 S.E.[2]	$\bar{x}$ errors ± 1 S.E.[3]
C-MS-48	8	30.1 ± 3.0	8.8 ± 1.0
C-MS-72	8	34.1 ± 2.9	9.0 ± 0.8
C-non-48	8	33.0 ± 2.6	10.1 ± 0.9
C-non-72	8	27.6 ± 1.3	8.3 ± 0.6
0.1-MS-48	8	29.6 ± 2.6	8.3 ± 1.1
0.1-MS-72	8	26.1 ± 1.7	7.5 ± 0.7
0.1-non-48	8	30.1 ± 1.8	9.3 ± 0.5
0.1-non-72	8	27.6 ± 3.4	8.1 ± 1.5
1.0-MS-48	8	23.0 ± 1.6	5.8 ± 0.6
1.0-MS-72	8	25.0 ± 1.9	7.6 ± 0.6
1.0-non-48	8	23.6 ± 2.2	6.6 ± 1.2
1.0-non-72	8	23.0 ± 1.0	5.4 ± 0.6

[1] N = number of fish per group.

[2] $\bar{x}$ trials ± 1 S.E. = mean number of trials to criterion plus or minus one standard error.

[3] $\bar{x}$ errors ± 1 S.E. = as above with number of errors to criterion.

group but both these groups were significantly different from the 1.0 tu group ($p < 0.01$). A high correlation was found between the number of trials and number of errors to criterion ($r = 0.9176$). A dose response curve is indicated (Fig. 4).

Part II.

a. Change in discriminating ability. In general, all controls improved or did not change from the pretest to the posttest while all the 0.8 tu fish showed a decrement in discriminating ability. The two intermediate groups, the 0.3 and 0.5 tu groups, were variable.

Due to the addition of an extra set I^2, which made unequal sample sizes in each of the four time periods, it was necessary to check for the effect of time on change in discriminating ability. Each of four doses were tested for the effect of time of testing by an ANOVA test. The controls, 0.3 or 0.8 tu groups did not show significant differences between the testing times; however, the 0.5 tu group showed significant differences ($F = 9.49$ at 3, 9df, $0.01 < p < 0.001$). The data from this group were further tested with a Duncan's Multiple Range Test which showed the 09:30 to 10:30 group to be significantly different from the 10:45 to 11:45 group ($p < 0.01$), but neither of these were significantly different from the other two testing times. Because of the small sample sizes involved (three or four in each time period), because time had no significant effect on any of the other dose levels, and because this one significant effect was between only two of the four times tested in the 0.5 tu group, it was decided to accept this deviation as a chance occurrence, ignore it, and pool the data from all four time periods in each dose.

Figure 4. Mean number of trials and errors to criterion for pooled data.

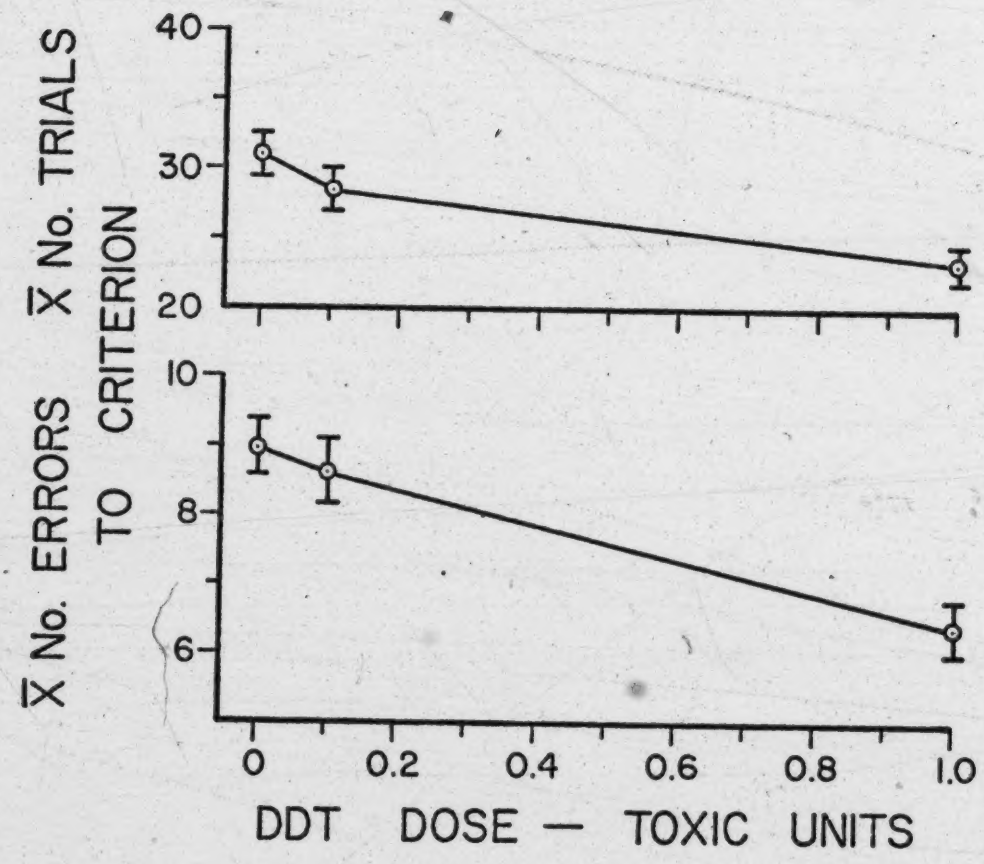

X̄ No. ERRORS X̄ No. TRIALS TO CRITERION
40
30
20
10
8
6
0
0.2
0.4
0.6
0.8
1.0
DDT DOSE — TOXIC UNITS

FIGURE 4.

The mean change in discriminating ability for each of the four doses is shown in Fig. 5. A Duncan's Multiple Range Test showed the control group to be significantly different from all other groups, the 0.3 and 0.5 tu group to be significantly different from all other groups but not from each other, and the 0.8 tu group to be significantly different from all others ($p < 0.01$). Thus a dose response curve is indicated; as the concentration of DDT increases, the ability of the fish to discriminate between two light intensities decreases.

b. Retention. Retention is the ability to retain or remember what has been learned. In order to look at the effect of DDT on retention, the number of errors made in the first 20 trials before and after DDT administration was compared. Because the order of presentation was constant in these 20 trials they were comparable. Only the first 20 trials were used as once the fish was shocked a few times the response observed would no longer represent retention but relearning. The retention score was taken as: Number of errors in the first 20 trials before DDT administration minus the number of errors in the first 20 trials after DDT administration. Again a positive number would indicate improvement. No correlation was found between dose and retention; in fact all retention scores are essentially the same (Table 5). A Duncan's Multiple Range Test showed no significant differences between retention scores ($p > 0.01$).

c. Performance. This generally means how many errors an animal makes in executing a well established response. Because no two fish gave exactly the same responses during discrimination testing

Figure 5. The effect of DDT on discriminating ability. The data used for this figure are in Appendix 4.

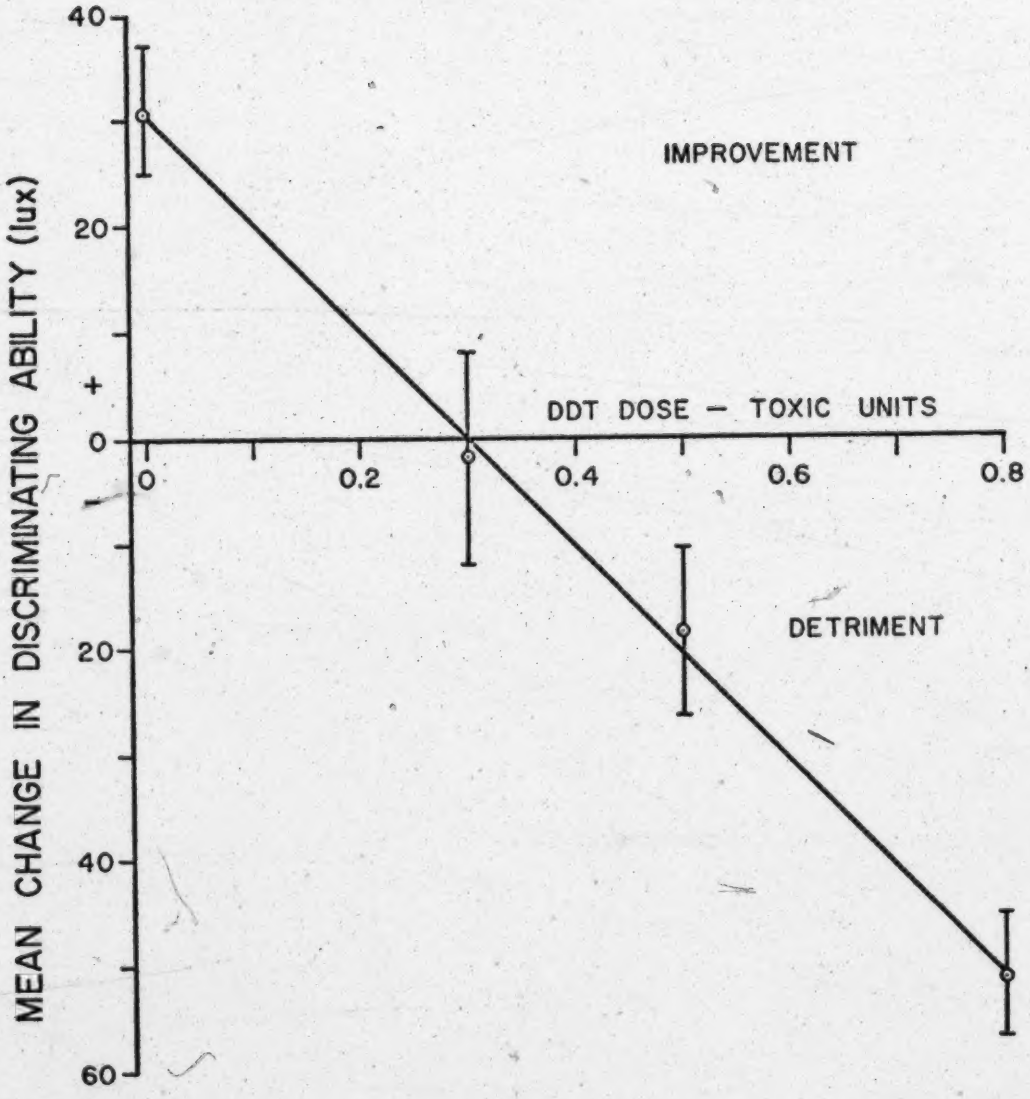

MEAN CHANGE IN DISCRIMINATING ABILITY (lux)
40
20
+
0
20
40
60
IMPROVEMENT
DDT DOSE — TOXIC UNITS
0
0.2
0.4
0.6
0.8
DETRIMENT

FIGURE 5.

Table 5. Retention scores (number of errors in the first 20 trials before DDT minus number of errors in the first 20 trials after DDT administration) for each of the four dose levels.

Group	N	Mean retention score ± 1 S.E.
Control	13	1.92 ± 0.8
0.3 tu	13	-1.31 ± 1.1
0.5 tu	13	-0.85 ± 0.8
0.8 tu	13	1.62 ± 0.8

and in fact no one fish responded the same in the pretest and posttest, the measurement of performance is subject to some variability. For example, a fish in its pretest might make only correct responses at 723-22 lux, 572-28 lux and 419-41 lux but have to repeat 273-64 lux three or four times until it made no mistakes at that level. In its posttest it might have to repeat 419-41 lux a number of times. The two are thus not fully comparable. Comparison of trials between fish showed the same kind of variability. It was not possible to eliminate this variability as all fish did not respond to the testing situation in the same way. In order to compensate for this, performance was expressed as a percentage. This was done as follows: for each fish at a given dose on a given day (i.e. either before or after DDT administration) both the number of errors and the total number of light presentations at a given level of paired light intensities were found. This was done for each of the levels above the level of discriminationg ability (i.e. 723-22 lux, 528-28 lux, 419-41 lux and 273-64 lux). Then the total number of errors and light presentations for each level above the level of discriminating ability were added up from all fish in a given dose level on a given day, and the fraction (number of errors over total number of light presentations) was converted to a percent, which represented the percent of errors shown by all fish at a given dose of DDT for one level of paired light intensities above the level of discriminating ability (Table 6). A comparison of the percent error (Table 6a) at each of the different pairs of light intensities shows much variability, both in comparison of percent error within a given dose, between doses, and before and

Table 6a. Performance scores (% error at each discrimination level). See text for explanation.

Group	Test	Paired Light Intensities (lux) 723-22 lux	528-28 lux	419-41 lux	273-64 lux
Control	pretest	19.50	18.95	10.38	9.69
	posttest	12.50	6.96	11.00	7.66
0.3 tu	pretest	6.42	15.26	10.22	14.87
	posttest	15.71	15.36	12.04	11.81
0.5 tu	pretest	10.71	8.23	16.42	12.07
	posttest	11.35	11.31	3.21	4.23
0.8 tu	pretest	17.85	13.77	13.80	14.88
	posttest	8.33	5.71	10.17	6.52

Table 6b. Averages of the % errors at all four paired light intensity levels.

Group	Pretest	Posttest
Control	14.63	9.53
0.3 tu	11.69	13.73
0.5 tu	11.85	7.52
0.8 tu	15.07	7.68

after DDT administration. The percent errors for each of the four light intensity levels were averaged (Table 6b) and the general trend emerges of an increase in performance between the pretest and posttest (before and after DDT treatment) indicated by a decrease in percent errors, in all doses except the 0.3 tu group. None of these changes were found to be significant with a Chi-Square test.

In summary, most of the doses tested showed a small non-significant increase in performance, so no effect of DDT is evident.

DISCUSSION

The results of the present study show that DDT caused an increase in learning rate (defined as the rate of acquisition of a conditioned response), a linear decrease in discriminating ability, and no change in retention or performance of the conditioned response. MS 222 was found to have no effect on learning ability with or without DDT.

The effects of DDT on learning as found in this study are not readily comparable to the mammalian literature. Khairy (1959) found that DDT had no effect on problem solving in rats (species not given). In this case the problem was a maze in which the pattern was changed each time; this is not learning. He also used an extremely small sample size (only three animals per group). Medved *et al.* (1964) in their review of the work of Serebrianaia (1955) say that DDT generally caused a lowering or "total cessation of the conditioned reflexes" (*op. cit.* 1964:58). However, this refers to the effect of DDT on an already established response; according to McGaugh and Petrinovich (1965), a drug can only influence a well established response by affecting performance and provides no information about the acquisition of the response. Al-Hachim and Fink (1968) tested in a shuttlebox the offspring of mice (species not stated) given DDT and found delayed acquisition of the conditioned avoidance response. However, this study suffered from only a limited range of DDT doses (pregnant mice all given 2.5 mg/kg body weight at one of three stages in pregnancy) and the use of food deprivation which can influence the toxicity of DDT.

The method of administration of DDT (from mother to foetus) was also not comparable to the present study. Yuhas (1970) concluded that rats (*Rattus norvegicus albinus*) showed no difference in learning with DDT, but he defined learning as a change in behaviour during a maintained performance of a task on a special schedule of reinforcement. This would be considered performance by McGaugh and Petrinovich (*op. cit.*). Sobotka (1971) showed that passive avoidance (defined as fear induced suppression of motor activity) in mice (species not stated) given DDT was decreased (i.e. they made more wrong responses). He also stated that DDT did not impair acquisition (this in direct contrast to the results of Al-Hachim and Fink *op. cit.*). Craig (1972) tested DDT fed mice (species not given) in a T maze and found that the mice at low doses took the same number of trials to reach criterion as controls while the highest dose (1.0 tu) required more trials. In terms of number of errors a biphasic response was seen; at low doses (approximately 0.4 tu) the mice made fewer errors than controls, at approximately 0.7 tu they were the same as the controls, and at 1.0 tu they made more errors than controls. No change in locomotor activity was observed so the changes were not due to a decreased ability to perform the tests. It is interesting to note the change in errors to criterion did not parallel the change in trials to criterion as it does in the present study. Craig (*op. cit.*) also states that some of the differences in results in the above studies may be due to the doses used; that is, if a low dose is used the results would show one part of the biphasic response, but if a high dose is used, the other part of the biphasic response would be seen. Often the relation between the dose used and the LD50

is not stated. In summary, the mammalian literature generally suggests no effect on or a decrease in the rate of acquisition of the conditioned response, in contrast to the present study which found an increase in the rate of acquisition of the response. It is doubtful if the studies are comparable, however, as learning processes may not be the same in the two classes of animals (e.g. Bitterman 1968).

More closely related to the present study are those studies dealing with the effect of DDT on learning in fish, particularly because most looked at a similar conditioned response. In the present study, using an oral dose of DDT, it was found that DDT at 1.0 tu significantly increased the learning rate, while DDT at 0.1 tu did not. Anderson and Prins (1970) showed that brook trout (*Salvelinus fontinalis*) exposed to sublethal DDT doses either could not be conditioned to exhibit the propeller-tail reflex or required significantly more trials than the controls. This simple conditioned response was used to eliminate any effects of sensory or motor impairment which could influence acquisition of a response requiring co-ordinated motor activity. However, because it is such a simple response in relation to the task learned in the present study, it may not be affected in the same way by DDT. Anderson and Peterson (1969) found that brook trout trained by electric shock to avoid the side of their preference in a two-chambered aquarium could not be conditioned if exposed to DDT at 20-60 ppb for 24 hr. However, Jackson *et al.* (1970) found this was mainly due to the water level in the apparatus and DDT treated fish could learn as well as the

controls if the water level were lowered. In fact they repeated the experiment done by Anderson and Peterson (*op. cit.*) and found that although the fish were not learning the correct response (i.e. going through the door into the next chamber when the light was turned on) they were learning a response. Unlike the controls, that escaped through the door, when the DDT treated fish were shocked they rose to the surface of the water and thus the probability of their going through the door was decreased. The DDT treated fish did learn to rise to the surface when the light came on but because this was not what the experimenters classified as learning, the fish were said not to have learned. Hatfield (1970) with a similar apparatus found that Atlantic salmon (*Salmo salar*) at the 1.0 tu DDT dose learned significantly better than controls, while those at the 0.1 tu dose did not. However, a dose response curve is indicated in Hatfield's (*op. cit.*) study. Although the 0.1 tu group was not significantly better than the controls, it did show "scattered" improvement (Hatfield and Johansen 1972) and the average number of trials to criterion was somewhat less than the controls (calculated from Hatfield and Johansen 1972 Fig. 2c). Thus the results of the present study agree well with those of Hatfield (1970). Direct comparison with Jackson *et al.* (1970) is not possible as the relation between the dose used and the toxicity is not given. A number of deaths occurred in Jackson's highest dose so perhaps it was near the LC50. Neither this highest dose or 1/10 that dose had any effect on learning (in terms of trials to criterion) in Atlantic salmon. No deaths occurred in the brook trout experiments so it is not possible to relate this to the LC50. The major difference between the

present study and that of Hatfield (*op. cit.*) is the use of an oral dose of DDT rather than DDT in the water.

Performance was not affected by DDT in the present study, the only one on fish. While Desi *et al.* (1966) and Yuhas (1970) found no effect of DDT on performance in rats, Craig (1972) showed a biphasic response, the low dosed animals making significantly fewer errors than controls and the mice with the highest dose making significantly more. Medved *et al.* (1964) indicate a general decrease in performance in cats with DDT. In the present study the mean performance of the fish at all doses but one improved non-significantly from the first to the second test but did not differ from each other in degree of improvement. Thus DDT had no effect on performance. Again the differences between learning both in and between mammalian and fish studies may be the reason for the difference in response. Performance might also have been measured in terms of time to reach the "goal", analagous to maze running time in mice. However, this is likely too subjective in the apparatus used, as the "goal" is a large area.

DDT has not been shown to affect retention of a conditioned response in the present study nor in any studies to date (Jackson *et al.* 1970; Sobotka 1971; Hatfield and Johansen 1972).

According to Anderson (1971) one of the effects of DDT poisoning in fish is a heightened sensitivity to stimuli. This is seen as a general increased sensitivity (Hatfield and Johansen 1972) or as a specific sensitivity to certain stimuli such as electric shock (Anderson and Prins 1970). However, Khairy (1959) suggests that this hypersensitivity (in rats) may be due to an exaggeration in

motor responses; thus the animals' responses to stimuli are exaggerated but sensitivity could remain unaltered. In those studies dealing specifically with the effect of DDT on discrimination, DDT has been found to decrease discriminating ability. The present study, the only one dealing with fish, showed a linear decrease in discriminating ability with increasing doses of DDT. Thompson and Lilja (1964) showed a transitory period of increased auditory acuity in rats given DDT "quickly" followed by a decrease. Davis (1965) found that DDT decreased the ability of bobwhite to discriminate between a red and green light. Dieldrin, another chlorinated hydrocarbon, has been shown to decrease auditory acuity in sheep (Elsberry 1973). Other investigators have attributed their results to a possible decrease in discriminating ability. Yuhas (1970) trained rats to respond when a light was turned on, not to respond when it was off. At high DDT doses the rats responded more in the light off period than before. This he suggested shows a change in discriminating ability. Hansen (1972) looking at the effect of DDT on salinity selection in mosquitofish (*Gambusia affinis*) suggested that changes seen as a result of DDT administration may reflect changes in the fish's ability to discriminate between different salinities. That DDT might specifically decrease visual sensitivity is suggested by the finding of Alderdice and Worthington (1959:48) that associated with a "high spray" of DDT to coho salmon (*Oncorhynchus kisutch*) was the development of blindness in the test fish. Therefore the present study would agree with suggestions in the literature in that DDT did decrease discriminating ability, although the dose response curve seen here is not evident in

any of the other studies except perhaps that of Thompson and Lilja (1964).

Exactly why DDT should affect these two parameters of central nervous activity - learning and discrimination - differently is not apparent. The present theory of learning or "memory storage" is that short term memory is based on transient neuronal processes, and that more permanent memory is based on further changes initiated or produced by these transient neuronal processes, such as RNA and protein synthesis (McGaugh and Petrinovich 1965). DDT has been shown to increase the frequency of impulses in nerves in a so-called "multiplication effect" (O'Brien 1967) and the frequency of brain waves. This overall increase in activity could result in an increase in the rate of learning, with all the activity being channelled into learning a specific response. This same increase in activity might also result in an increased sensitivity (as reported above) which would increase discriminating ability. The latter was not found to be true. Perhaps due to the overall increase in activity the response to fairly similar stimuli becomes the same, i.e. each stimulus produces so many impulses in the nerve that they are indistinguishable. This would explain the results of the present study but not one where thresholds were changed (e.g. auditory thresholds changed in Thompson and Lilja 1964). Looking at the number of impulses produced in the optic nerve with and without DDT administration at the various light intensities might indicate which part of the sensory system is involved. Perhaps the multiplication effect is not constant, so that a given impulse might multiply five times under one condition and only four at another. This would

result in the same stimulus giving a different response at two different times. Roy (vide Khairy 1959) found that high DDT doses caused bursts of impulses in the sensory nerve of an appendage of the cockroach which were interrupted by periods of no impulse in response to a stimulus; this type of response would certainly cause high light intensities to be read as lower. The effect may reside in the eye itself, although the paper by Alderdice and Worthington (1959) is the only one in which blindness in response to DDT is noted. DDT may affect bleaching of the visual pigments or accommodation. If DDT does obscure vision at the receptor level, the overall increase in response of the sensory nerve could be irrelevant. Due to changes in brain patterns, the stimuli may also be received differently and hence not read in the same way by the brian. Because learning involves mainly central nervous function while discrimination is based mainly on a response to sensory input, the two may be differently affected by DDT, especially if, as O'Brien (1967) says, the sensory nerves are the most sensitive to DDT and are the basis of its lethality (at least in insects).

A second explanation for the results in Part II of this study, rather than a direct effect on discrimination itself, might be an effect on short term memory. Because the lights were presented alternately, at 20 sec intervals, it may have been necessary for the fish to recall the intensity of the previous light in order to compare it to the light being shown. This would be a type of short term memory. Alternately the fish might have viewed each light presentation as an entity and compared it not to the previous light but rather to

the light intensities to which it had been trained. This would involve long term memory or retention.

Short term memory has been shown to be affected by DDT. Westlake and Kleerekoper (1970) discuss why the turning rate in fish can be considered memory and although they don't specifically label it as such it is very likely short term memory. The memory involved with turning rate has been shown to decrease with DDT (Davy and Kleerekoper 1970; Davy *et al.* 1972). Therefore if a fish used short term memory in the discrimination testing, it might show impaired discriminating ability due to a decrease of short term memory with DDT. It is very unlikely, however, that this effect would only be seen at levels near the limit of discriminating ability. Thus the effect of DDT on short term memory should be seen at all light intensity levels, which would result in an overall decrease in performance . This was not found to be true. Therefore it is likely that long term memory is being used, which was shown not to be affected by DDT, and that any effects of DDT seen on discriminating ability are actually effects on discriminating ability and not on memory. This conclusion suggests a further explanation for the decrease in discriminating ability. If a fish does compare each light with the "hypothetical" intensities, the increase in the number of nerve impulses for a given stimulus (the multiplication effect) due to DDT would cause low light intensities to be read as high, and high intensities as brighter than the hypothetical bright. So for example if a fish initially could discriminate at 238-80 lux but not at 200-96 lux, it would be seeing 238 lux as a bright light and 80 lux as a dim one. When given DDT, there will be

an increase in the frequency of impulses from all stimuli. Now the light at 80 lux appears bright, so both lights appear bright and the fish treats them as the same. His discriminating ability has decreased.

The light intensity range used in the present study was within the range of normal light intensities found in nature. Malinin (1970) found that the illumination at 3m in a low transparency lake at noon on a sunny day was approximately 500 lux; this decreased to 1 1/2 to 2 lux at sunset. Trout tend to live in fairly clear streams (high transparency) often in shallow ripples where the light intensity could easily reach the 700 lux used in the present study. However, the chief feature of light in rivers is the great variability in light intensity (Westlake 1966) so it is important to assess whether a change in discriminating ability in the fish of only 51 lux (in the 0.8 tu group) is of any significance. As Sprague (1971) points out, it is easy to document relatively small changes within an animal but it is often questionable whether the changes are deleterious or merely within the normal range of adaptation of the organism. The small decrease in discriminating ability seen in the present study could have deleterious effects in natural conditions. Although there is great variability in light intensity in rivers this variability is generally daily or seasonal and to some extent spatial. It is likely important for the fish to distinguish small changes in light intensity on a minute to to minute basis, as they could indicate for example the shadow of a predator overhead or a prey below. Visual discrimination of other things, such as distinguishing food from rocks, is also important to the fish. Thus the small changes in discriminating ability seen in

the present study could well be important for survival of these fish.

As well as its effect on learning and discrimination DDT has also been shown to affect other central nervous system functions, such as the cold block temperature (Anderson and Peterson 1969) and temperature selection (Ogilvie and Anderson 1965; Javaid 1967; Gardner 1973; Peterson 1973) in fish.

The present study showed that the anesthetic MS 222 does not influence learning either 48 or 72 hrs following administration at a level commonly used for anesthetization, 100 ppm (Wedemeyer 1970 used 80 ppm; Houston *et al.* 1971 used 100 ppm), either alone or with DDT. Studies by Wedemeyer (1970) and Houston *et al.* (1971) for example show diverse physiological effects of MS 222 but no work has been done to date concerning its effects on learning. Goddard *et al.* (1974) found behavioural changes in fish exposed to MS 222. When MS 222 treated fish were placed in a horizontal temperature gradient, they spent most of their time at the bottom, unlike the controls, and their temperature selection was less precise than controls. On the basis of these results they recommend that anyone using MS 222 prior to behavioural studies should allow at least one week to avoid after-effects of the anesthetic. The results of the present study contradict this suggestion; MS 222 showed no effect on learning as little as 48 hr after anesthetization. The difference between Goddard's and the present study may be due to the degree of anesthetization of the fish; those in Goddard *et al.*'s (*op. cit.*) study were anesthetized to "Stage 3 or Stage 4" while those in the present study to Stage 1 (as in Houston

and Woods 1972). This may explain the more permanent effects observed in the former study. The former study also looked at a very different kind of behaviour from the present study, although both involve central nervous function. It would seem likely that effects of MS 222 would disappear quickly, as turnover rates are very rapid, with 90% blood clearance after 55 min at 5 C (Houston and Woods 1972) and total clearance at 8 hr at 12 C (Hunn *et al.* 1968). Because of the widespread use of MS 222 as an anesthetic both in research and in hatchery practise (Schoettger *et al.* fide Wedemeyer 1970) and the equally widespread appearance of DDT and other chlorinated hydrocarbon residues in fish (e.g. Reinert 1970; Henderson *et al.* 1971), the lack of interaction between these two substances, at least in terms of learning rate, may prove important to fish biologists.

In nature, either the food chain (Macek and Korn 1970) or the water (Grzenda *et al.* 1970) may be the more important source of DDT to fish. The effects of water borne DDT have been extensively studied; the use of an oral dose in the present paper has looked at a previously unexamined aspect of DDT effects. The results of feeding fish a single, large dose of DDT given in the manner indicated in this paper would be analagous to a fish eating large numbers of insects which have been killed by spraying with DDT (Surber 1946; Ginsburg 1947; Hoffman and Linduska 1949; Hoffman and Surber 1949; Johnson 1963). Due to the persistence of the DDT in the fish (Gakstatter and Weiss 1967; Macek *et al.* 1970), the effect would still be apparent some time later. This is seen to some extent in the present study where similar effects of DDT on learning rate were seen 48 and 72 hr after

treatment and in the effect on discrimination 71 hr after treatment.

Although the present study involves the effect of a specific substance - DDT - on a number of parameters, learning, discrimination, retention and performance - its implications are far reaching. DDT was used as a representative of a class of compounds - the chlorinated hydrocarbons - which are widely used as pesticides. Many are similar in structure to DDT and may well have similar effects. The specific behavioural responses studied are representatives of the myriad behavioural responses an organism can show. All four of the responses studied are important to the survival of the organism. The fact that DDT enhances learning ability is offset by the fact that this enhancement is significant only at a dose which kills half the population; the benefits to an individual are outweighed by the detriment to the population. A decreased ability to discriminate could have broad implications; a fish which cannot distinguish food from non-food, a predator from a rock, or a foe from the shadow of a plant would not survive long. Because organisms have adapted specific behavioural traits through natural selection, these traits are likely the most efficient way to deal with the environment, and any changes in behaviour could be detrimental to members of a species.

SUMMARY

1. Rainbow trout (*Salmo gairdneri*) treated with 0, 10 or 100% of the 96 hr-LD50 dose of DDT (0, 0.1 and 1.0 toxic units (tu) respectively) were trained to perform a simple conditioned response, that of turning right in a T maze in response to a bright light. They were force fed DDT treated food pellets with or without MS 222 anesthetization, and tested 48 or 72 hr later. Learning rate, or speed of acquisition of the conditioned response, was measured in terms of both the number of trials and the number of errors to criterion. Time of testing and use of anesthetic had no effect on learning rate, while both number of trials and number of errors to criterion were significantly lower in the 1.0 tu group than in the control fish. Although learning rate was not significantly lower in the 0.1 tu group than in controls, a dose response curve is indicated. Thus DDT treated fish learned faster than the controls.

2. A second series of rainbow trout were trained to discriminate between a bright (723 lux) and a dim (22 lux) light. The fish were individually tested to find the minimum difference between light intensities which they could distinguish, given DDT at one of four levels (0, 0.3, 0.5 and 0.8 tu) and retested 71 hr later. The "change in discriminating ability" was found. The fish in the two tests were also compared for retention and performance of the task. All controls showed an increase in discriminating ability, and as the dose increased the fish showed a linear decrease in discriminating ability. There was no effect of DDT on retention or performance.

3. The effect of DDT on the four parameters - learning, retention, performance, and discrimination - are discussed in relation to the literature and possible explanations of the positive effect of DDT on learning as opposed to the negative effect on discriminating ability are considered.

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Appendix 1. The light intensities in lux measured under water at the position from which the fish normally viewed the light behind barrier B. These data are graphed in Figure 3.

Light switch setting	Dial setting	Light intensity (lux)
High	100	723
	90	572
	80	419
	70	273
	65*	238
	60	200
	55*	160
	50	127
Low	50	139
	45*	118
	40	96
	35*	80
	30	64
	20	41
	10	28
	0	22

*Extrapolated from Fig. 3.

Appendix 2. Data from lethality tests used to determine the 96 hr-LD50.

a. Preliminary tests using a broad range of DDT doses.

DDT dose mg DDT/gm body weight	Number dead after 96 hr / Total tested Set 1	Set 2	Total	% dead
0.05	2/3	3/5	5/8	63%
0.10	3/3	5/5	8/8	100%
0.20	2/3	5/5	7/8	88%
0.50	1/3	5/5	6/8	75%
1.00	3/3	5/5	8/8	100%

b. LD50 tests using a range below 0.05 mg DDT/gm body weight.

DDT dose mg DDT/gm body weight	Number dead after 96 hr / Total tested Set 4	Set 5	Set 6	Total	% dead
0	1/5	2/5	0/18	3/28	11%
0.02	-	-	5/18	5/18	28%
0.03	3/5	2/5	5/18	10/28	36%
0.04	3/5	3/5	12/19	18/29	62%
0.05	3/5	4/5	-	7/10	70%
0.06	3/5	3/5	-	6/10	60%

Appendix 3. Mean number of trials and errors to criterion for pooled data at each dose. These data are graphed in Fig. 4.

Group	N	$\bar{x}$ trials ± 1 S.E.	$\bar{x}$ errors ± 1 S.E.
Control[1]	32	31.2 ± 1.3	9.0 ± 0.4
0.1 tu[1]	32	28.4 ± 1.2	8.3 ± 0.5
1.0 tu[1]	32	*23.7 ± 0.8	*6.4 ± 0.4

[1]Control = pooled data for all controls, 0.1 tu = pooled data for all 0.1 tu groups etc.

*Significantly different from controls, $p < 0.01$.

Appendix 4. Change in discriminating ability before compared to after DDT administration, change in lux. These data are graphed in Fig. 5.

Group	N	Mean change ± 1 Standard error
Control	13	30.8 ± 6.2
0.3 tu	13	-1.9 ± 10.6
0.5 tu	13	-18.5 ± 8.0
0.8 tu	13	-51.3 ± 5.8